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14. ABSTRACT

1. Engineer a 1-step injection technology to modify the cartilage-fluid interface to reduce friction and ultimately joint degradation. We will leverage our new HA-binding peptide technology to functionalize normal and arthritic cartilage surfaces to localize and bind the synovial lubricant and biofunctional molecule HA, a current mainstay in clinical practice today to reduce pain and delay surgery.
2. Translate the HA-binding technology to preclinical joint models of post-traumatic osteoarthritis. The one-step injection of the HA-surface binding technology will be tested in a model of a common meniscal injury where osteoarthritis develops without treatment. The ability of the technology to reduce the progression, prevent, and treat osteoarthritis caused by joint destabilization will be determined.

15. SUBJECT TERMS

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Engineered Joint Lubrication for OA Prevention and Treatment

1. Introduction

The overall goal of this translational project is to design a cartilage surface treatment to prevent, mitigate, and treat osteoarthritis (OA) induced by trauma. OA is characterized by progressive loss of cartilage on the surface of the joints. Instead of a smooth, lubricating cartilage surface at the end of bones in the joint, a loose, fibrillated cartilage tissue develops that eventually is lost altogether. The military and veteran population is particularly susceptible to OA due to the high demands of physical exercise and extreme environments [1-5]. As such, the rate of OA is much greater in the military population compared to the civilian. The articular joint and related injuries are complex with aspects of tissue structure loss, inflammation and lubrication all playing roles in dysfunction and disease. While cartilage repair is one key component in joint reconstruction, engineered cartilage does not have the lubrication properties of native tissue and overall joint lubrication is disrupted in disease. Therefore, we are now taking a more comprehensive approach to treat joint dysfunction and have developed a new technology to improve the interface of the cartilage tissue surface with synovial fluid and also therapeutic hyaluronic acid (HA) therapies (Fig 1). To address the challenge of treating joint dysfunction and prevent/reduce the development of post-traumatic osteoarthritis using our new technology we propose to:

1. Engineer a 1-step injection technology to modify the cartilage-fluid interface to reduce friction and ultimately joint degradation. We will leverage our new HA-binding peptide technology to functionalize normal and arthritic cartilage surfaces to localize and bind the synovial lubricant and biofunctional molecule HA, a current mainstay in clinical practice today to reduce pain and delay surgery.

2. Translate the HA-binding technology to preclinical joint models of post-traumatic osteoarthritis. The one-step injection of the HA-surface binding technology will be tested in a model of a common meniscal injury where osteoarthritis develops without treatment. The ability of the technology to reduce the progression, prevent, and treat osteoarthritis caused by joint destabilization will be determined.

Ultimately, the proposed research here targeting joint lubrication integrates with our research (and others in the field) focused on reconstructing cartilage and related tissues and using small molecule drugs to target inflammation, to create a comprehensive approach for treating the complex joint environment and its dysfunction. The project is translational in nature and will leverage the PI's translational experience, including a repair strategy to treat focal cartilage defects that has been tested now in over 40 patients along with clinical trials for related soft tissue technologies. The proposed joint lubrication strategy to reduce post-traumatic osteoarthritis program is responsive to the PROP FY13 Focus Area of "Novel Interventions for Strategies to Prevent, Mitigate, or Treat Post-traumatic Osteoarthritis resulting from fracture and/or ligament injury."

2. Keywords

- Osteoarthritis
- Lubrication
- Biomaterial
- Post-traumatic
- Hyaluronic acid
- Knee
- Cartilage
- Prevention
- Intra-articular
- Binding
- Injection

- Pre-clinical

3. Accomplishments:

- **What were the major goals of the project?**

Specific Aim 1: Engineer normal and arthritic cartilage surfaces with the HA-binding peptide technology to improve surface lubrication and HA localization. Specifically, the one-step HA-binding peptide-polymer technology will be applied to normal and osteoarthritic cartilage explants from bovine model and human sources. Chemical analysis will be performed to confirm tissue modification and mechanical analysis will be performed to evaluate friction and lubrication properties in tissue explants and rodent articular joints.

Milestone 1. Define the chemistry for one-step injection of HA-binding-polymer conjugate that optimally binds the cartilage surface for translation. This aim is completed.

Specific Aim 2: Engineer normal and arthritic cartilage surfaces with the HA-binding peptide technology. Specifically, the one-step HA-binding peptide-polymer technology from Aim 1 will be applied to normal and osteoarthritic cartilage explants from bovine model and human sources. Mechanical analysis will be performed to evaluate friction and lubrication properties in tissue explants.

Milestone 2. Confirm binding of technology to normal and arthritic cartilage tissue and define functional changes in tissue lubrication properties with treatment. This aim is 75% finished.

Specific Aim 3: Translate the HA-binding technology to preclinical joint models of post-traumatic osteoarthritis.

Milestone 3. Demonstrate in vivo functionality of HA-binding technology and outcomes of therapy in treating posttraumatic OA in a preclinical rodent model. This aim has been started but not completed.

Accomplishments

1. Completed radioactive labeling of PEG-COLBpep in order to characterize bio-distribution.
2. Tested binding efficacy of multiple HA binding peptides in reducing OA in an anterior cruciate ligament (ACL) mouse model of OA.

Reportable Outcomes

1. We successfully labeled and imaged PEG-COLBpep with indium.
2. We successfully implemented multiple HA binding peptides in the mouse OA model.
3. Surface binding of HA-binding peptides were directly confirmed using quartz crystal microbalance dissipation (QCM-D).

Progress Detail

This year we addressed Subtask 1 and 2 of Specific Aim 3.

Subtask 1: Lifetime of radioactively labeled peptide (figure 1)

The goal of subtask 1 was to determine the lifetime of radioactively labeled HA binding peptide in the joint. To simplify this task, the PEG-COLBpep moieties were indium labeled without the HABpep moiety. This allowed PEG to be tagged instead of the HABpep moiety, which could potentially be hydrolyzed in the knee joint and complicate the radioactivity measurement.

Labeled PEG-COLBpep was injected into the mouse knee (n=5) and imaged using VECTOR (1mm resolution). For each mouse, 5-6 images of the knee were acquired at the following time points: five,10, and 15 minutes, one hour, three hours, six hours, and 24 hours. Images were analyzed in amide and pixels were correlated to µCis based on a standard. Radioactivity was plotted against time and two separate half lives were determined: a distribution half life and an elimination half life. Elimination half life corresponds to the kinetics of drug elimination from the body, and elimination half life of the PEG-COLBpep in the knee (based on uCi content time 3-24hrs) is on average 33hrs (standard deviation: 20 hrs).

It is possible that the lifetime of the PEG-COLBpep injected in the study will be increased when the entire HABpep-PEG-COLBpep is injected because binding to HA would in theory help retention of the molecule.

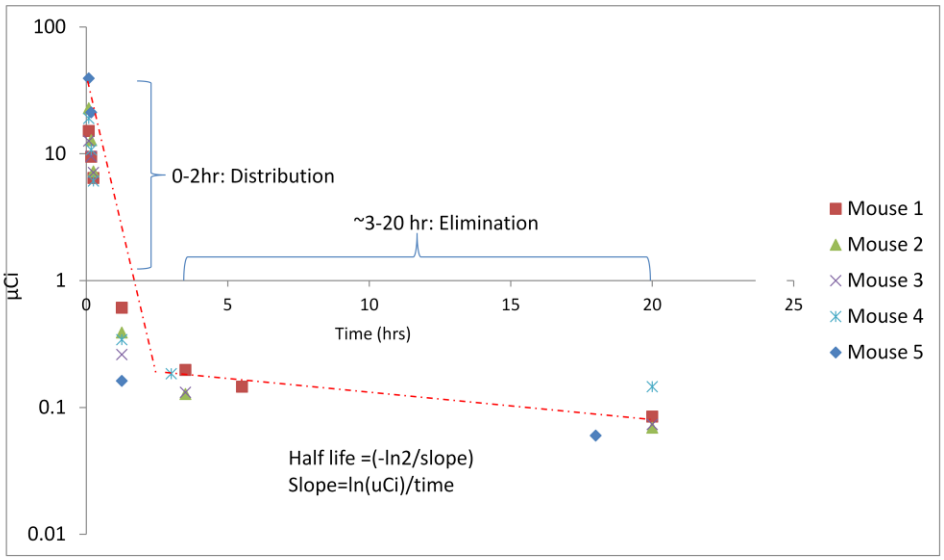


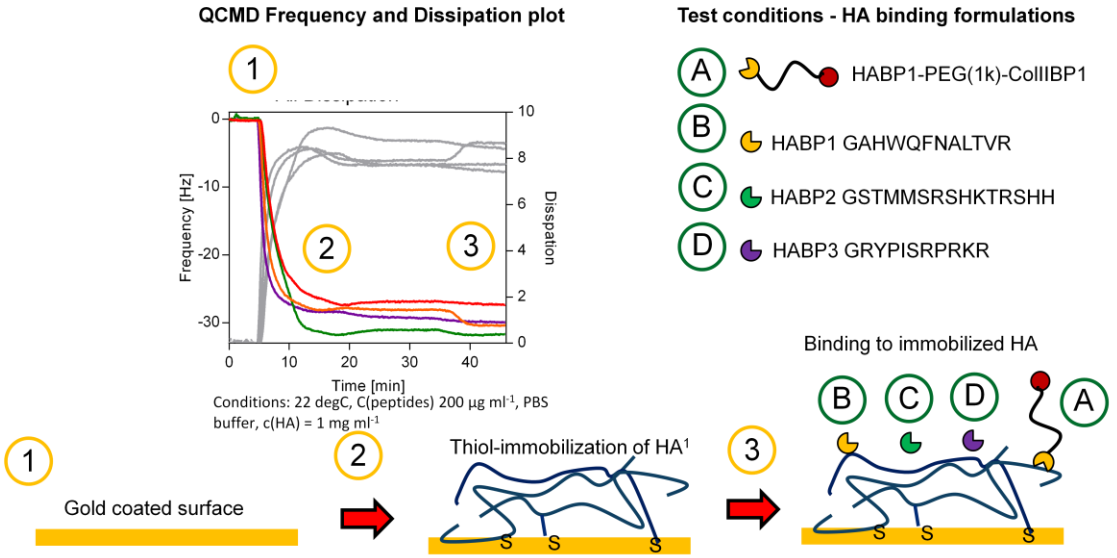
Figure 1: Radioactivity of indium labeled PEG-peptide. Labeled PEG-peptide was injected into the mouse knee (n=5) and imaged using VECTOR. Based on the uCi activity in the knee, we estimate that the elimination half life of the peptide in the knee (based on uCi content time 3-24hrs) is on average 33hrs (standard deviation: 20 hrs).

Subtask1: Screening of HA binding peptides for affinity to thiol-immobilized HA

There are currently several HABpeps and PEG lengths available and the formulation of the HA-binding-polymer conjugate that optimally binds the cartilage surface and HA needs to be determined. To do so, binding of different HABpeps and PEG lengths in the HA-binding treatment are being compared.

The binding of several HA-binding proteins to cartilage surfaces via Quartz crystal microbalance with dissipation monitoring (QCM-D) were compared. Hyaluronic acid was thiol-immobilized onto a QCM-

D chip surface and coated with different formulations of peptide to determine peptide ability to bind HA. Additionally, peptide was applied to collagen coated QCM-D chips to determine COLBpep ability to bind collagen 2. Through these experiments, HABpep constructs were established to bind to immobilized HA and to collagen 2 (figure 2). There are still more PEG moieties to be tested.



[1]Majd, Sara Ehsani, et al. "Both Hyaluronan and Collagen Type II Keep Proteoglycan 4 (Lubricin) at the Cartilage Surface in a Condition That Provides Low Friction during Boundary Lubrication." *Langmuir* 30.48 (2014): 14566-14572.

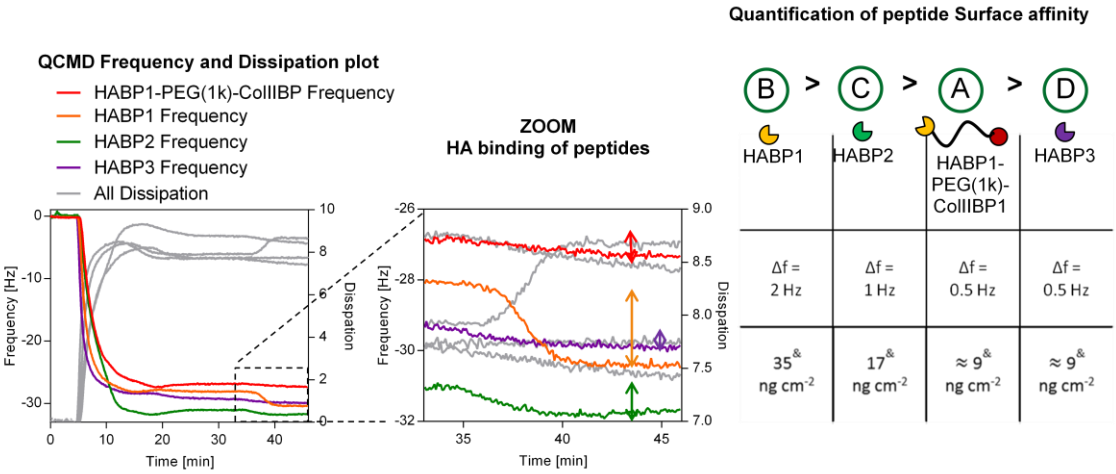


Figure 2: Quartz crystal microbalance is used to demonstrate binding of knee-joint related substrate, in this case thiol-immobilized hyaluronic acid. We can quantify binding of HABP composition using the frequency change converted into mass per surface are. We will continue screening and fine-tuning of these promising composition on Collagen I and II substrates using full constructs

Subtask 2: Reduction of OA development in an anterior cruciate ligament transection model of OA using the HA-binding treatment

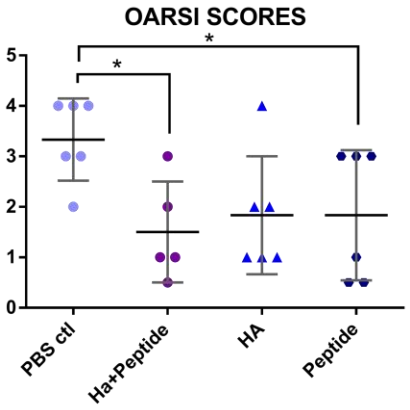
An anterior cruciate ligament (ACL) transection model of OA is being used because it is commonly used and known to induce OA 4 weeks after surgery. Mechanical models such as this are

advantageous because there is a fast and reproducible time course of disease progression and a clear cut relationship between the OA-initiating event and the development of pathology.

In order to test the ability of HA-binding protein to reduce OA, the following treatment groups were selected for comparison: 10µL of 20mg/mL HA, 10µL of 10mg/mL HA-binding protein 1, and 10µL of HA and HA-binding protein 1 (in three separate animal groups). Each treatment was injected intra-articularly 2 weeks after ACL transection. The mice were euthanized 4 weeks post ACL transection in order to compare induction of OA in treated and untreated samples. The outputs from this experiment include histology, quantitative PCR, and functional pain tests.

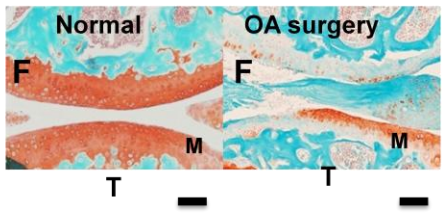
In histology, the OARSI OA Histopathology Assessment system was used to evaluate the severity of OA. All treatment groups were found to provide some improvement to the cartilage surface including peptide alone (figure 3a). Additionally, quantitative PCR was used to evaluate inflammatory, matrix degrading, and cartilage related genes. Both the HA binding peptide 1 and HA+ HA-binding protein 1 groups had statistically significant decreases in MMP13 compared to the PBS control (figure 3b). These groups also show lower IL-6 and TNFα (although not quite statistically significant). Nevertheless, the qPCR and histology results are encouraging.

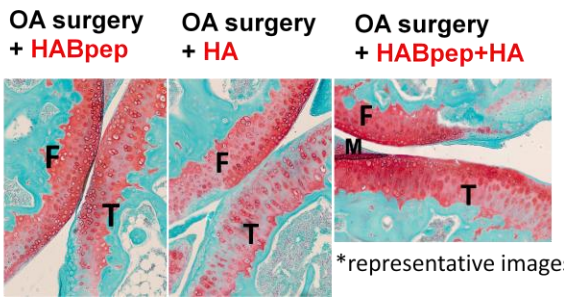
A.



Statistics: Mann Whitney
**PBS vs HA: p value= 0.06

Safranin O staining*:





*representative images

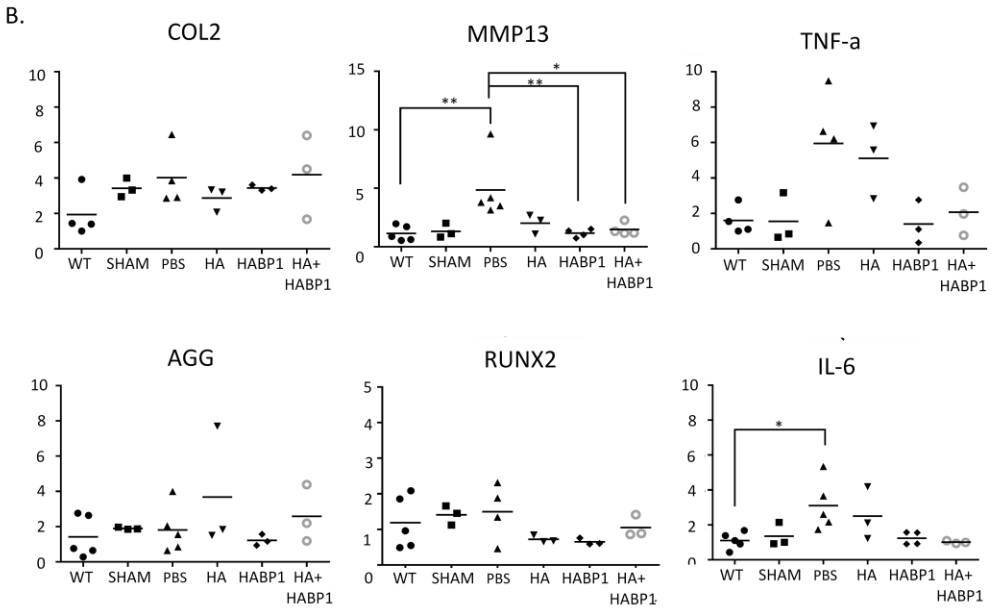


Figure 3: A. Reduction of OA in the murine ACL post traumatic OA model. 2 weeks after surgery, mice were treated with a 10uL intra-articular injection of either PBS, 10mg/ml peptide, 20mg/ml of HA, or both HA and peptide. 2 weeks after injection, mice were sacked and knees collected for histology. We observe a statistically significant reduction in OA using the peptide (based off of OARSI scoring of the histology). B. quantitative PCR indicating lower levels of MMP13 in HABP1 and HA+HABP1 treatment groups.

Subtask 2: Reduction of OA development in an anterior cruciate ligament transection model of OA using the HA-binding treatment: Comparison of peptides *in vivo*

In order to test the ability of different HA-binding protein to reduce OA, the following treatment groups were selected for comparison: 10µL of 20mg/mL HA-binding protein 1 (HABpep1 on graph), 10µL of 50mg/ml HA-binding protein 2 (HABpep2), and 10µL of 50mg/ml HA-binding protein 3 (HABpep3). HA-binding protein 1 was limited to a 20mg/ml concentration because that is the maximum solubility of the construct.

In histology, the OARSI OA Histopathology Assessment system was used to evaluate the severity of OA. All peptides were found to provide some level of improvement to the cartilage, although none are statistically significant due to low numbers (this was a pilot study for the HABpeptides) (figure 4). Additionally, functional pain tests were performed at baseline (before surgery) and two and four weeks post ACL transection. The pain tests include the hot plate test, in which a mouse is placed on a hot plate and recording the paw-lick response time. The weight bearing test is also used, in which mice stand in a chamber and weight placed on each of the hind legs is recorded. Pain reduction was seen with treatment of HABpep2 and HABpep1 (20mg/ml).

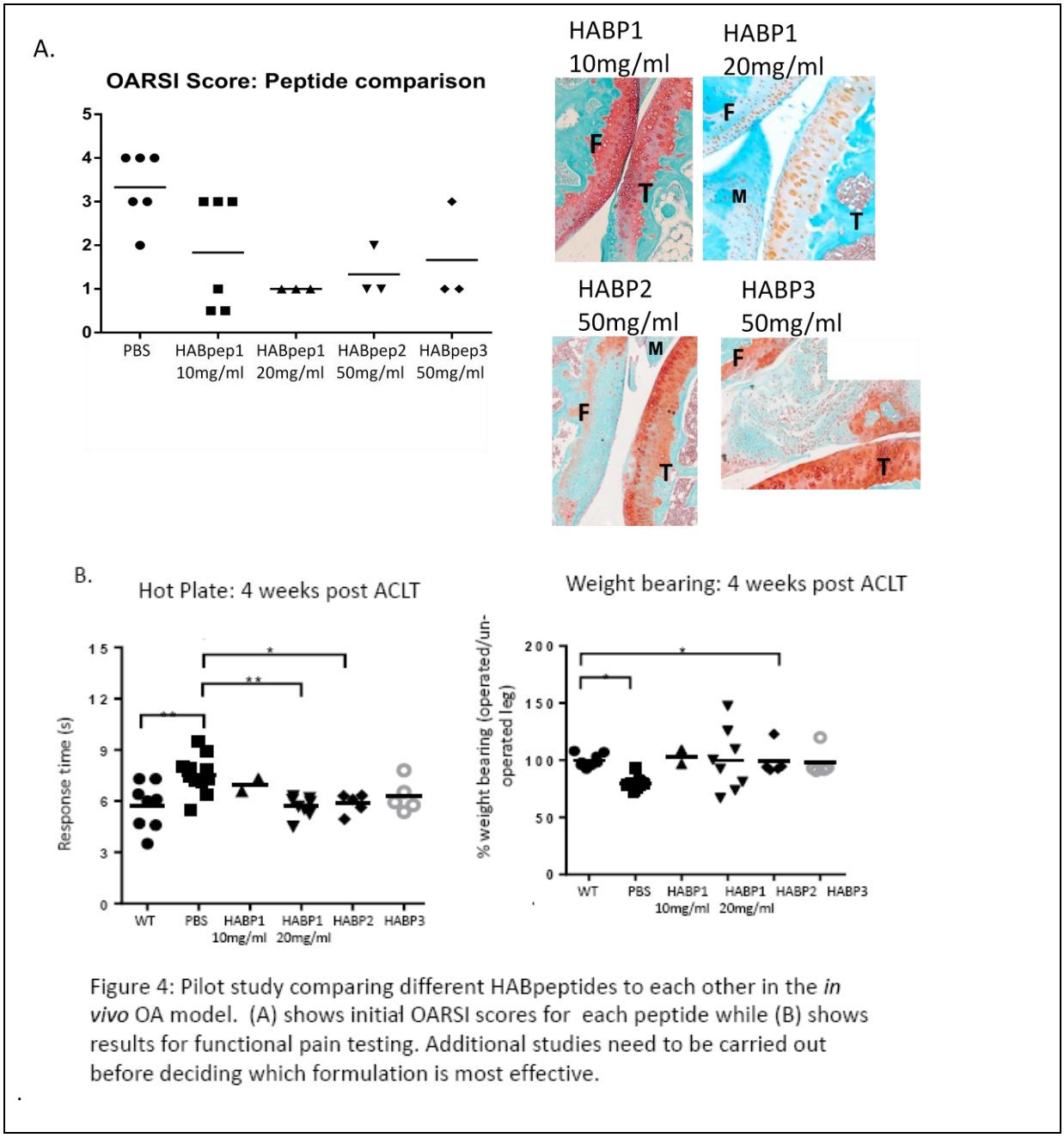


Figure 4: Pilot study comparing different HABpeptides to each other in the *in vivo* OA model. (A) shows initial OARSI scores for each peptide while (B) shows results for functional pain testing. Additional studies need to be carried out before deciding which formulation is most effective.

What opportunities for training and professional development has the project provided? The project has provided PhD students to travel to two conferences, one on hyaluronic acid and another on osteoarthritis.

How were the results disseminated to communities of interest? Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

1. Compare surface binding of more peptide formulations using QCM-D on knee-joint related substrates: collagen I, II, HA for further optimization. This will include comparison of an 8-arm peg formulation with each HABpep (1,2,3).
2. We will test more controls and peptide formulations and dosages in the functional OA mouse model, including the 8 arm peg.
3. A large cohort will be run in which the optimal peptide, concentration, and number of injections will be run.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The results of this research will likely have an impact on understanding the progression of post-traumatic osteoarthritis in our murine OA model.

What was the impact on other disciplines? There is potential for this technology to be used for lubricating the eye, which applies to the study of dry eye.

What was the impact on technology transfer? Nothing to report**.**

What was the impact on society beyond science and technology? The results from this project, if translated into the clinical setting, will have the potential to improve everyday life of people living with osteoarthritis.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them Nothing to report.

Changes that had a significant impact on expenditures Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents Nothing to report.

Significant changes in use or care of human subjects Nothing to report.

Significant changes in use or care of vertebrate animals. Nothing to report.

Significant changes in use of biohazards and/or select agents Nothing to report.

6. PRODUCTS:

Journal publications:

Singh, A., et al., *Enhanced lubrication on tissue and biomaterial surfaces through peptide-mediated binding of hyaluronic acid.* Nat Mater, 2014. **13**(10): p. 988-95.

Conference Abstracts:

1. **Jacobs,H.;** Singh, A.; Rathod, S.; Sommerfeld, S..; Elisseeff, J.; HYALURONIC ACID BINDING PEPTIDE-POLYMER SYSTEM FOR TREATING OSTEOARTHRITIS. *Poster*. (2015) International Society for Hyaluronan Sciences, Florence Italy.

Patents:

Biomaterials comprising hyaluronic acid binding peptides and bifunctional biopolymer molecules for hyaluronic acid retention and tissue engineering applications. **Publication number:** 20140369975

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Personnel	Position	Role	Percent Effort
Heather Jacobs	PhD student	OA functional tests, Radioactivity	55%
Ani Singh	Post Doc	Radioactivity	15%
Micheal Corvelli	Researcher	uCT	5%
Sona Rathod	Graduate Student	OA functional tests	20%
Sven Sommerfeld	Post Doc	Binding studies	5%

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** *Nothing to Report.*

- **What other organizations were involved as partners?**
    - **Location of Organization:** *JHMI, Pomper Group*
    - **Partner's contribution to the projec**t
        - **Collaboration**: Members of the Pomper Group helped in radioactive tagging of the peptide.

8. SPECIAL REPORTING REQUIREMENTS
QUAD CHART:

Engineered Joint Lubrication for OA Prevention and Treatment

OR130378

GRANT 11452424

Year End Report (2015)

PI: Jennifer Elisseeff **Org**: Johns Hopkins University School of Medicine **Award Amount**: $1,215,000

Product Aims this Year (2015):

1. To synthesize peptide-polymer conjugates.
2. Characterize the lubrication properties of peptide-polymer treated and untreated cartilage samples via rheology.
3. To complete radioactive labeling of PEG-COLBpep with Indium 111.
4. To compare surface binding of multiple peptide formulations using QCM-D

Reportable outcomes this Year:

Specific Aim 1, subtask 1:
We synthesized PEG-peptide conjugates.
Specific Aim 1, Subtask 2: Different PEG-peptide conjugates were studied for HA binding efficiencies and capabilities using QCM-D, HABP1 was found to have good binding affinity.
Specific Aim 2, subtask 1: Rheometer studies completed and peptide-polymer was found to reduce frictional properties of cartilage (subtask 2).
Specific Aim 3, Subtask 1:
Bio-distribution studies determined that the elimination half life of the PEG-COLBpep from the knee was approximately 30 hours.

Timeline and Cost:

Activities	2014	2015	2106	2017
Synthesis of polymer-peptide conjugate and chemical analysis				
Frictional analysis of the tissue lubrication				
Injection and retention in the articular joint				
Test for prevention and treatment of post traumatic osteoarthritis				
Estimated Budget ($K) direct	$125	$250	$250	$125

Goals/Milestones met this year:

1. Made and characterized peptide polymers and tested rheological properties on cartilage.
2. Completed radioactive labeling of PEG-COLBpep in order to characterize bio-distribution.
3. Validated peptide distribution via Indium labeling of PEG-COLBpep.

Plans for next year:

1. Compare surface binding of more peptide formulations using QCM-D to aid in peptide-polymer selection.
2. To test HA binding peptide efficacy in reducing OA in an anterior cruciate ligament (ACL) mouse model of OA.

9. APPENDICES

Included:

Full publication of: Singh, A., et al., *Enhanced lubrication on tissue and biomaterial surfaces through peptide-mediated binding of hyaluronic acid.* Nat Mater, 2014. **13**(10): p. 988-95.

ARTICLES
PUBLISHED ONLINE: 3 AUGUST 2014 | DOI: 10.1038/NMAT4048
nature materials

Enhanced lubrication on tissue and biomaterial surfaces through peptide-mediated binding of hyaluronic acid

Anirudha Singh[1], Michael Corvelli[1], Shimon A. Unterman[1], Kevin A. Wepasnick[2], Peter McDonnell[1] and Jennifer H. Elisseeff[1]*

Lubrication is key for the efficient function of devices and tissues with moving surfaces, such as articulating joints, ocular surfaces and the lungs. Indeed, lubrication dysfunction leads to increased friction and degeneration of these systems. Here, we present a polymer–peptide surface coating platform to non-covalently bind hyaluronic acid (HA), a natural lubricant in the body. Tissue surfaces treated with the HA-binding system exhibited higher lubricity values, and *in vivo* were able to retain HA in the articular joint and to bind ocular tissue surfaces. Biomaterials-mediated strategies that locally bind and concentrate HA could provide physical and biological benefits when used to treat tissue-lubricating dysfunction and to coat medical devices.

Lubrication, a process that reduces the resistive force between two opposing surfaces and decreases friction and wear[1], is key to the function of a number of industrial technologies, including vehicular engines, wind turbines and hard drives[2]. Here, chemists join with engineers to design surfaces that work together with liquid lubricants to achieve low-friction systems. Lubrication between tissues is also important to maintain low-friction movement within a number of biological systems, including the pleural cavity, the surface of the eye, visceral organs, and diarthroidal joints[3]. In diarthroidal joints, healthy, painless movement is facilitated both by molecules at the tissue surface and in the lubricating synovial fluid. Synovial fluid bathes the joint surface with several molecules that contribute to lubrication, including lubricin[4,5], surface-active phospholipids[6–8] and HA (ref. 9). The role of each of these components has been supported and challenged on the basis of various *in vitro* studies on cartilage lubrication[9]; however, in a healthy joint, these molecules work synergistically and with the tissue surface to reduce friction coefficients and to achieve normal physiological performance[9–11]. Similarly, in the eye the ocular surface and tear fluid should work synergistically[12], and insufficient lubrication on the ocular surface is a key component of dry-eye disease. Medical devices employed in certain tissues, such as contact lenses for ocular surfaces, generally lack lubrication, hampering their integration with the body and their function. Today, therapeutic options to enhance tissue and device lubrication focus only on replacing or enhancing the lubricant in the fluid phase, severely limiting functional capability and longevity. Taking cues from industrial applications and the normal tissue–fluid interface, we designed a biomimetic system for tissue and biomaterial surfaces to work synergistically with fluid-phase biological lubricants.

To develop a biomimetic surface modification, HA-binding peptides (HABpep), discovered through phage display[13–17], were covalently or non-covalently bound to surfaces through a hetero-bifunctional poly(ethylene glycol) (PEG) chain. The HABpep–polymer system non-covalently binds HA, either endogenously available in the local fluid environment or provided exogenously, to the modified surface. We reasoned that localizing HA to tissue surfaces through HABpep would provide the physical benefits of increasing both cartilage lubrication to the cartilage and retention of HA in the articular joint. Binding HA to the cartilage tissue mimics one of the functions of the lubricin protein[18] that is normally found on the surface of cartilage and that is altered during disease[19].

In addition to its lubrication role, HA has a number of potential biological functions that would be ideal to concentrate at material and tissue surfaces, including reducing inflammation, mediating matrix-metalloproteinase expression and protecting cells from free-radical damage[20–22]. Coating surfaces with HA may also physically protect the surfaces from cytokines and degrading enzymes that are frequently found in a diseased, post-traumatic or surgical environment. Finally, and most critically, the presence of the polymer–HA-binding modification provides a biomimetic mechanism to concentrate HA on the surface. Numerous endogenous enzymes and reactive oxygen species can degrade HA, and its fluid concentrations can quickly decrease with normal turnover through local transport processes[22]. The HABpep can capture HA that is found in low concentrations in a diseased environment or lost through a physical or biological mechanism, and provide the stable anchor on the tissue surface that is necessary to dynamically bind and concentrate HA where it is needed.

HA binding to cartilage

The HA-binding strategy was first applied to treat the surfaces of osteoarthritic cartilage (Fig. 1a). As osteoarthritis is so common and debilitating, there is a significant interest in understanding and treating the disease. Joint lubrication is one of the key disease components that may be addressed to improve overall joint health and reduce the progression of cartilage degeneration[6]. Healthy articular cartilage provides low-friction properties to the synovial joint through a combination of lubrication mechanisms, including interstitial fluid pressurization and boundary lubrication[9]. Pressurized fluid, within the tissue and between the surfaces, can bear significant levels of load. In addition, lubricant molecules, such as HA, lubricin and phospholipids on the articular cartilage surface, provide functions that include boundary lubrication and mediating

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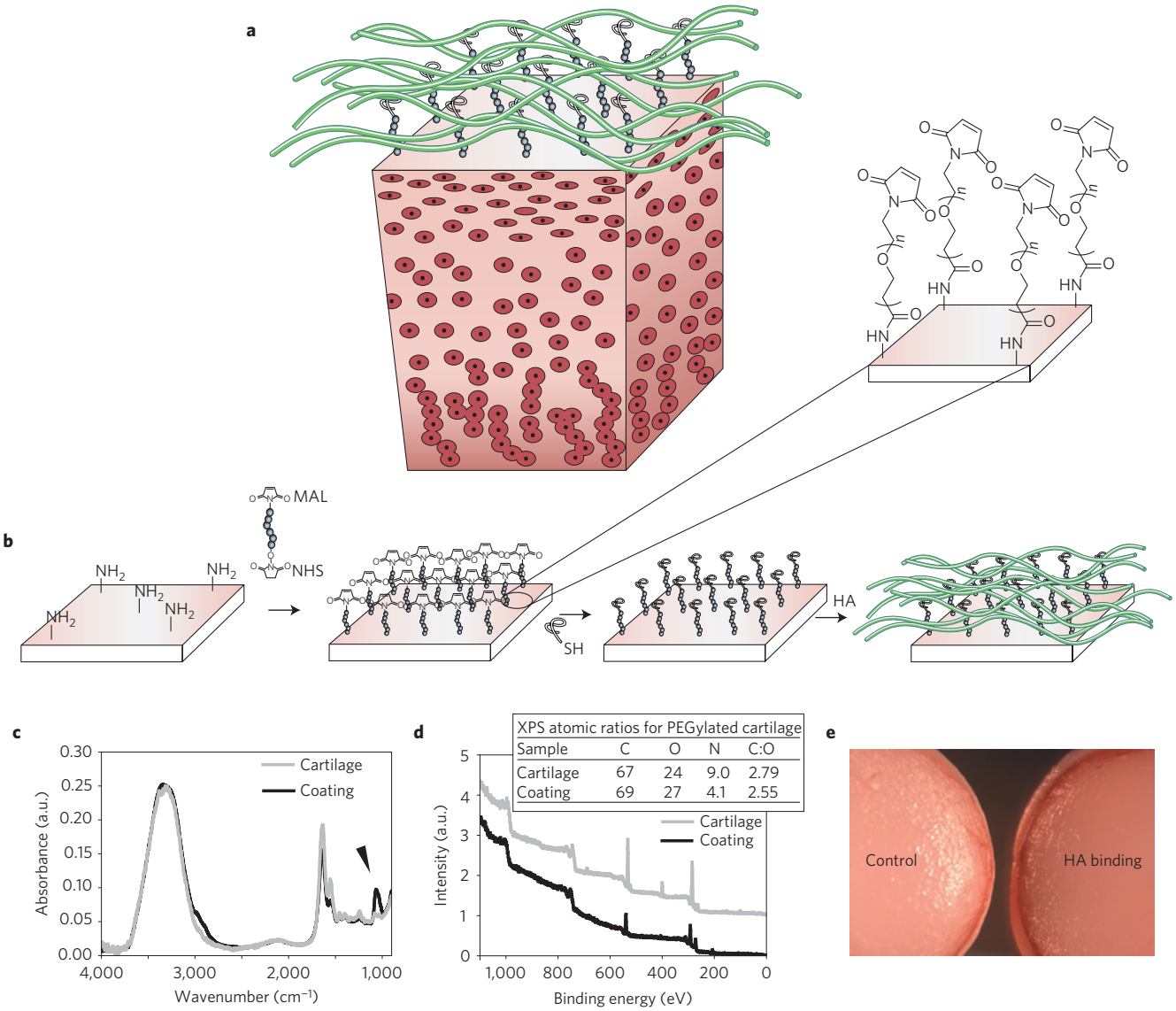

Figure 1 | Tissue-surface modification with a HABpep–polymer system. a, Schematic of a cartilage surface modified with a HABpep designed to interact with and bind HA in surrounding fluid. **b**, An *in vitro* covalent strategy for coating the cartilage surface with MAL–PEG–NHS crosslinker, which on reaction with primary amines of the cartilage surface creates an exposed thiol-reactive surface. Subsequently, a thiolated HABpep is reacted to the maleimide functionality. On exposure to a HA solution, the HA binds to the peptide–polymer coating on the cartilage surface. **c**, The PEG crosslinker reaction to articular cartilage was confirmed by ATR-FTIR spectroscopy, which validated the presence of the ether-rich PEG coating with a large ether peak at ~1,066 cm^{-1}. **d**, PEGylation was further verified by XPS atomic ratios. Compared with unmodified cartilage, coated samples had a carbon to oxygen ratio closer to 2 (the ratio in PEG) and significantly lower nitrogen content. **e**, HA-binding functionality of the peptide-conjugated cartilage was visualized using a biotinylated HA. Biotinylated HA was synthesized and applied to unmodified cartilage and cartilage modified with the HA-binding polymer system. After thorough washing, the biotinylated HA was treated with streptavidin and horseradish peroxidase for visualization. The tissue surfaces treated with the HA-binding polymer coating stained darker than the untreated native cartilage.

load bearing[8,9]. Osteoarthritic knees are challenged by altered and abnormal structural and compositional changes that include depleted and disrupted boundary lubricants[23,24]. Unless addressed, these changes can further increase surface friction, accelerate degeneration of cartilage, and lead to abnormal joint motion.

HA is a component of the native cartilage, tissue matrix and is present in the synovial fluid. HA in the synovial fluid works synergistically with the tissue surface and molecules such as lubricin to provide wear protection and improve joint lubrication, in addition to biological functions[18,25,26]. For example, high-molecular-weight HA reduced cartilage degeneration in a rabbit model, possibly through restoration of the viscoelastic properties of synovial fluid[27–29]. In addition, researchers have suggested potential biological functions of HA in the joint in *ex vivo* models, such as protecting chondrocytes from oxidative stress, reducing inflammation and promoting chondrocyte cell survival[30–32]. As a result, one common clinical treatment for osteoarthritis is injection of HA directly into the joint to improve synovial lubrication. Despite the importance of HA in the normal joint and its attributes, clinical results of HA injections, also known as viscosupplementation, have been controversial, with some clinical studies showing benefits but others not achieving statistically significant efficacy[33–36]. This lack in efficacy of injectable HAs may be due in part to the clearly observable rapid turnover of HA molecules within the joint after injection, the use of non-physiological crosslinked HA, and the limited ability to target the regions of tissue where

increased lubrication is needed. Although we have previously targeted cartilage reconstruction[37,38], engineered cartilage lacks normal lubrication properties[6], and the tissue surface is an ideal focus for therapeutic intervention to prevent the development of osteoarthritis and to treat existing disease by protecting the underlying tissue from exposure to physical and biological elements.

To create a HABpep coating, a bifunctional PEG (*N*-hydroxysuccinimide–PEG–maleimide (NHS–PEG–MAL)) was attached to the amine-rich tissue surface through an amine–NHS reaction, which was further linked to HABpep through a MAL–thiol reaction (Fig. 1b). PEGylation of the cartilage surface coating was confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Polymer-modified surfaces produced spectra that indicated a large peak at ~1,066 cm^{-1}, consistent with the ether bonds of the PEG spacer that was not present on unmodified cartilage (Fig. 1c). In addition, X-ray photoelectron spectroscopy (XPS) spectra of the PEGylated cartilage compared with native cartilage demonstrated a significant decrease in nitrogen content and a drop in the carbon-to-oxygen ratio (closer to 2, the ratio in PEG), indicating that a synthetic, lower-nitrogen-containing layer had been successfully grafted to the cartilage surface (Fig. 1d). HABpep functionalization on the tissue explant and its ability to bind HA was confirmed by positive staining on incubation with biotin hydrazine-labelled HA and streptavidin-conjugated horseradish peroxidase (Fig. 1e). Direct covalent binding, although useful for device coatings, will be difficult to implement clinically.

Non-covalent tissue binding

Considering the translation of materials strategies to *in vivo* applications, we developed an additional chemical strategy to introduce the surface coating in a single-step application that can be employed alone or with existing viscosupplementation technologies. In contrast to targeting amine groups of the cartilage tissue with a reactive functionality (for example, NHS), we targeted the collagen molecules of the tissue surface as an anchor for the HABpep coating. A collagen-binding peptide (ColBpep) was employed to non-covalently bind HABpep to collagen in tissues, creating a coating of HABpep on the surface. A thiolated PEG spacer was linked to the HA-binding peptide (Fig. 2a), followed by the Michael-addition reaction of thiol functional groups with vinyl dimethyl azlactone. PEG with HABpep and azlactone functionality was further conjugated to a peptide that non-covalently binds to the molecules present in the target tissue, for example, collagen II present in the articular cartilage. ^{1}H-NMR and FTIR-ATR spectra (Supplementary Fig. 1a,b) confirmed the functionalization of PEG with azlactone (~2.9 ppm of CH_2 of thioether and ~1,750 cm^{-1} of FTIR-ATR spectrum) and collagen-binding peptide (6.5–7.5 ppm of ^{1}H-NMR spectrum). This methodology is based on a specific non-covalent interaction that does not undergo hydrolysis; therefore, HA can be mixed with HABpep–PEG–ColBpep before application. The azlactone-based HABpep system can also be used for *in vivo* covalent reactions with amine functionalities of biomaterial or tissue surfaces, and does not release any side products on ring opening of lactone by amines. Furthermore, azlactone's superior hydrolytic stability[39] compared with NHS allows mixing of the polymer–HABpep with an aqueous solution before injection.

In vivo targeting

Translation of the tissue-surface modification strategy to the complex *in vivo*, and, specifically, the joint environment, is required for therapeutic application. The half-life time of HA retention after intra-articular joint injection can vary from a few hours for ~10^6 Da (~17 h) to ~9 d (crosslinked HA with ~mol wt 23×10^6 Da), depending on the molecular weight of injected HA and the injection site[36,40,41]. However, purported HA-induced positive effects in joint pain and joint function are reported to last for months, highlighting the potential multiple mechanisms of action[41]. We applied the HABpep–polymer to rat joints to evaluate *in vivo* efficacy of HA binding and retention in a complex environment. In a single step, a mixture of HABpep–polymer (50 µl) designed to target Type II collagen (10.0 mg ml^{-1}) and fluorescently labelled HA (20.0 mg ml^{-1}) was injected into rat knees. Time-course imaging of fluorescence demonstrated that the HABpep–polymer coating improved HA retention in rat joints compared with controls with no surface modification. Surface treatment in combination with the HA injection increased longevity of linear HA in the joint over 12-fold (6 h in controls to 72 h in surface-treated joints, Fig. 2b). Through specific, non-covalent interactions, the HA was anchored to the tissue surfaces of the rat knee through Col IIBpep and the HABpep bridge. Although lubricin is present in the normal joints tested, these *in vivo* experiments (Fig. 2b) demonstrated that bound HA through HABpep was retained for longer times in normal rat knees (up to 72 h) compared with control without HABpep (6 h). Therefore, even in the complex and harsh environment of the knee, the polymer–peptide binding system increased retention of linear HA in the joint, prolonging the potential physical and biological benefits.

Frictional properties

As the physical lubrication properties of HA are a key therapeutic modality of function in the joint, we further investigated the impact of surface modification. In particular, we compared this to the lubricity ($\langle\mu\rangle_{\text{in PBS}} - \langle\mu\rangle_{\text{in HA}}$) of cartilage treated with the surface modification (surface-bound HA only) versus untreated cartilage in a bath of HA. Cartilage samples were then coated with HABpep–PEG–Col IIBpep polymer, pretreated with HA, thoroughly washed to remove unbound HA, and mechanically tested in phosphate-buffered saline (PBS) (Fig. 3a and experimental set-up pictured in Supplementary Fig. 2c). The static and kinetic total-friction values for normal cartilage tissue decreased significantly (35% and 72%, respectively; Fig. 3b,c) when tested in a HA bath ($\langle\mu_s\rangle$ of 0.018 and $\langle\mu_k\rangle$ of 0.008) compared with a PBS bath ($\langle\mu_s\rangle$ of 0.028 and $\langle\mu_k\rangle$ of 0.028). With application of the HABpep coating and HA pretreatment, the cartilage samples tested in PBS recorded an $\langle\mu_s\rangle$ of just 0.014 and an $\langle\mu_k\rangle$ of 0.008, levels similar to the HA bath (Fig. 3b,c). Normal cartilage treated with HA-binding coatings and pre-incubated in HA was able to replicate the low-friction characteristics of native cartilage tested in a HA-rich environment. This pivotal result suggests that most of the lubrication effects of HA on the tissue can be replicated by surface-bound HA alone, without the need for large concentrations of HA in the local environment. HABpep technology consistently reduced total friction values that include physiologically relevant and reproducible elements of interstitial-fluid depressurization and boundary-layer lubrication mechanisms (Supplementary Information and Supplementary Fig. 3).

Diseased tissue represents a further challenge, as it is characterized by a rough, fibrillated surface with very different frictional properties. Multiple pathogenic mechanisms can lead to cartilage deterioration and not necessarily correlate to higher friction values[42], including inflammatory and other biochemical pathways. The results from our mechanical testing however suggested that *in vitro* degenerated cartilage exhibited higher friction values compared with normal samples using the cartilage-on-cartilage mechanical-testing protocols (Supplementary Information and Supplementary Fig. 2). A number of recent studies also found a similar correlation of increasing osteoarthritis stage and increasing frictional response[43,44]; however, the increased potential for osteoarthritic cartilage pressurization may be considered a factor. Tribological studies of diseased human cartilage (osteoarthritic) explants, categorized by severity of cartilage damage, suggested that HA improved the lubrication properties of the diseased tissue to an even greater degree

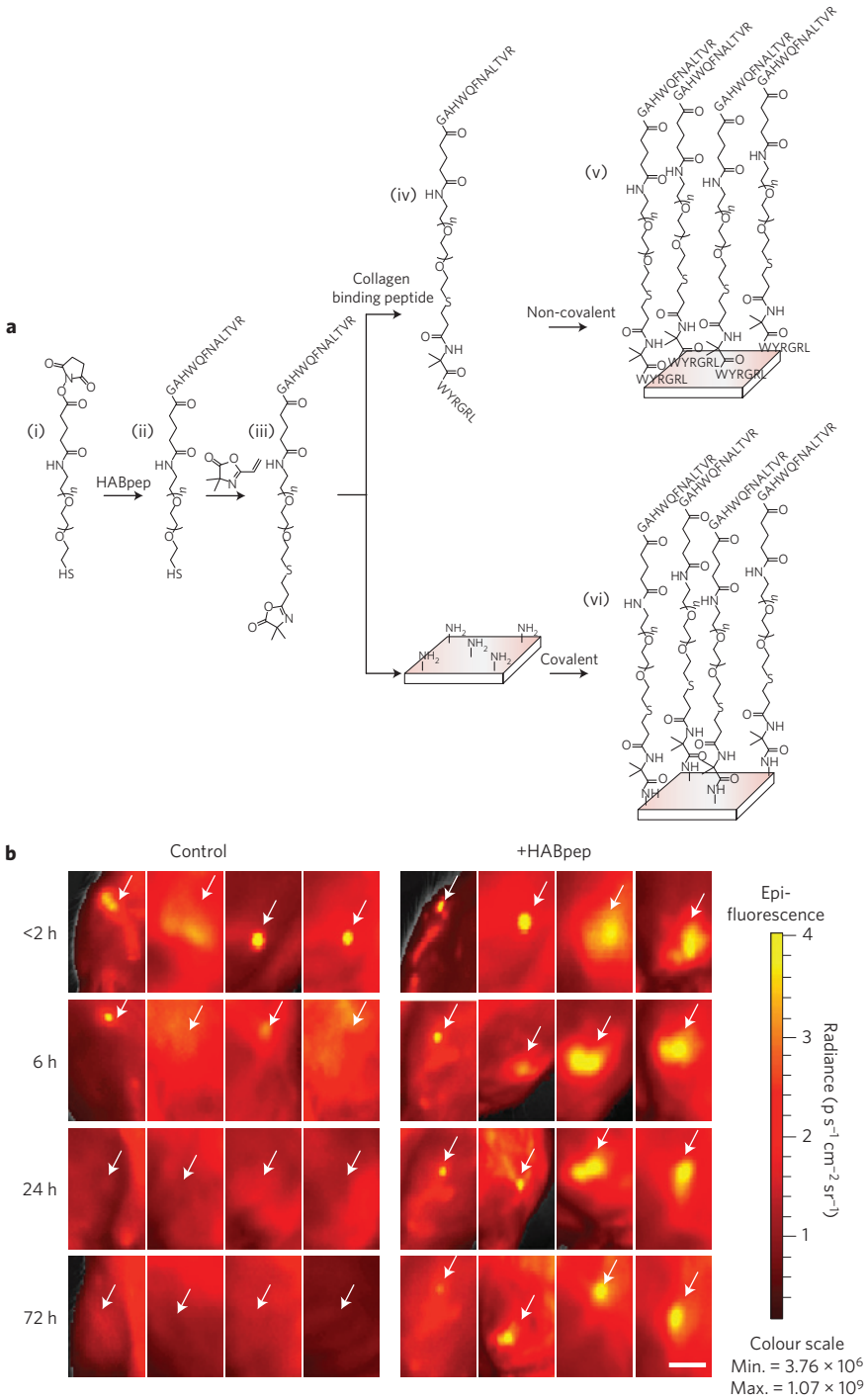

Figure 2 | Single-step strategy for the application of the HABpep–polymer system to a tissue surface, and functional translation to a joint environment. **a**, Schematic of the synthesis of a PEG bifunctional linker with one end group as a HABpep (GAHWQFNALTVR) and another end that either reacts with the amine groups or binds to a tissue surface through an extracellular matrix (ECM)-binding peptide, such as collagen II binding peptide, WYRGRL. First, a HA-binding peptide is linked to a thiol–PEG–SGA linker (i) through an amine–SGA conjugation reaction (ii), followed by the Michael-addition reaction of thiol functionality and vinyl dimethyl azlactone (iii). On a tissue surface, this amine-reactive azlactone functionality can be conjugated with either a peptide (iv) that non-covalently binds to ECM components (v), or covalently reacts with the amine functionality present in the tissue (vi). Both (iii) and (iv), with or without HA, can be applied on a tissue surface in a single-step application. **b**, HA–rhodamine together with HABpep–PEG–Col IIBpep was injected into healthy rat knees in a single step, and HA retention was monitored over time using an IVIS spectrum *in vivo* imager. HA–rhodamine (white arrows) through the HABpep–polymer system was retained in rat knees even 72 h post-injection, compared with only 6 h without HABpep coating. Scale bar, 1 cm.

than those of normal tissue. An increasing HA-lubrication effect was observed to be proportional to the severity of osteoarthritic cartilage (Supplementary Fig. 2u,v). The static and kinetic lubricity values for the most severely damaged osteoarthritic samples were approximately 5 times higher (Supplementary Fig. 2w,x) than those of the normal 'healthy' cartilage samples. These values directly relate to the surface damage and low lubrication values in osteoarthritic cartilage samples and highlight the need and increased benefit for

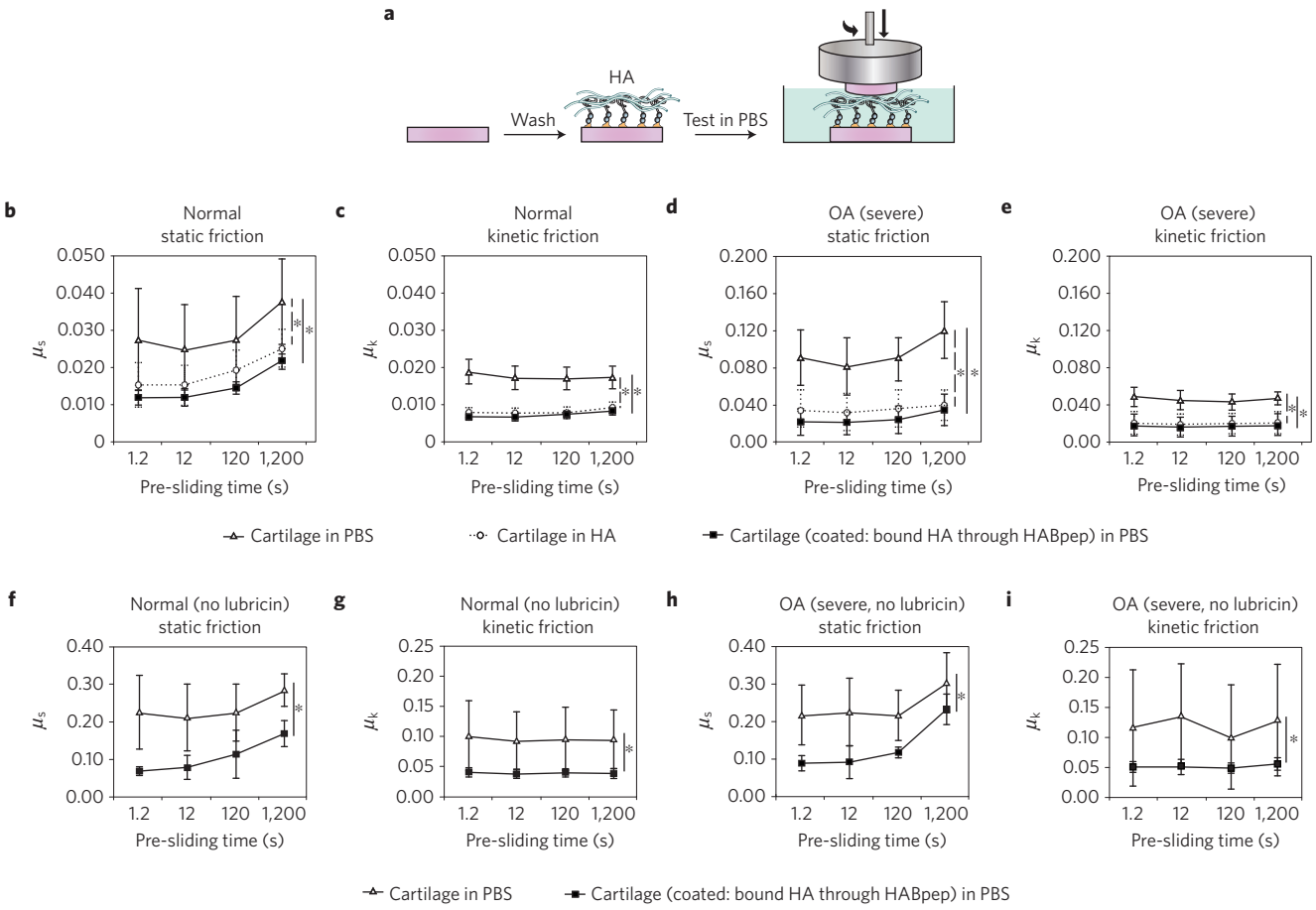

Figure 3 | Cartilage-surface-bound HA through the HABpep–polymer coating system in the absence of an exogenous lubricant can recapitulate the friction coefficients of high-concentration HA lubricants. a, Representative schematic for the preparation and incubation of HABpep-coated samples in test solution PBS. Lubrication properties of normal cartilage and severely damaged cartilage coated with the polymer–peptide system were tested in the presence of saline, and compared with uncoated surfaces in either saline or HA. **b**–**e**, Representative graphs of static friction and kinetic friction versus pre-sliding time (s) for the normal cartilage sample (**b**,**c**) and severely damaged cartilage sample, osteoarthritis (OA) stage 3–4 (**d**,**e**). The error bars indicate mean ± standard deviation; broken lines represent cartilage samples (no HABpep modification) in PBS versus HA bath, and solid lines represent cartilage samples in PBS versus cartilage samples coated with bound HA through HABpep in PBS; one-way analysis of variance Tukey's test, $^{*}p \leq 0.05, n = 3$. Cartilage-surface-bound HA through the HABpep–polymer coating system reduced friction values when lubricin is extracted from the tissue. The lubrication properties of normal cartilage and severely damaged cartilage (lubricin removed) coated with the polymer–peptide system were measured in PBS and compared with controls. **f**–**i**, Representative graphs of static friction and kinetic friction versus pre-sliding time (s) for the normal cartilage sample (**f**,**g**) and severely damaged cartilage sample, osteoarthritis stage 3–4 (**h**,**i**); the error bars indicate mean ± standard deviation; solid lines represent cartilage samples (lubricin removed) in PBS versus cartilage samples (lubricin removed) coated with bound HA through HABpep in PBS; unpaired *t*-test with the correction method, $^{*}p \leq 0.05, n = 3$.

enhanced lubrication and delivery of HA to the diseased environment. Similar to the results from the normal cartilage surfaces, the osteoarthritic cartilage samples treated with HABpep–polymer coating that produces surface-bound HA produced static and kinetic friction values nearly equal to those found with testing in a HA bath (Fig. 3d,e). Osteoarthritic cartilage samples with the HABpep–polymer coating and bound HA had higher static and kinetic lubricity values compared with normal modified tissue (Supplementary Fig. 4a,b), which suggests that the HABpep and bound HA have a greater effect on improving the lubrication of rough osteoarthritic surface compared with their effect in normal tissues. The practical implication is that even in a pathological environment, where low HA levels are present in the synovial fluid[31], the HA-binding coating can concentrate the limited HA available at the tissue surface to improve lubrication. Both normal and arthritic cartilage tissue benefited from the application of the HA-binding technology with respect to lubrication and HA retention in the articular joint, suggesting that the technology is useful even in the presence of lubricin or could be synergistically applied with lubricin[25,26]. To further evaluate functional capabilities, the HA-binding technology was applied to normal and arthritic cartilage tissues that were treated to remove lubricin. Surface-bound HA through the HABpep–polymer coating system significantly reduced friction on normal ($\langle\mu_s\rangle$ of 0.23–0.12 and $\langle\mu_k\rangle$ of 0.12–0.047) and osteoarthritic tissue ($\langle\mu_s\rangle$ of 0.24–0.13 and $\langle\mu_k\rangle$ of 0.12–0.051), confirming that the technology functions both in the presence and absence of lubricin (Fig. 3f–i). As HA bound to the tissue surface coating was washed vigorously before testing (Supplementary Fig. 2a), the improved lubrication implies that a relatively stable surface coating of HA is generated on the tissue and that will not be quickly flushed from the joint. Fluorescence imaging of the cartilage explants pre- and post-mechanical testing also verified the retention of HA–rhodamine onto the surface (Supplementary Fig. 4c). Semi-quantitative analysis of surface fluorescence found that HABpep–polymer increased binding of HA compared with no treatment. HABpep–polymer increased surface binding on normal tissue and tissue treated to remove lubricin

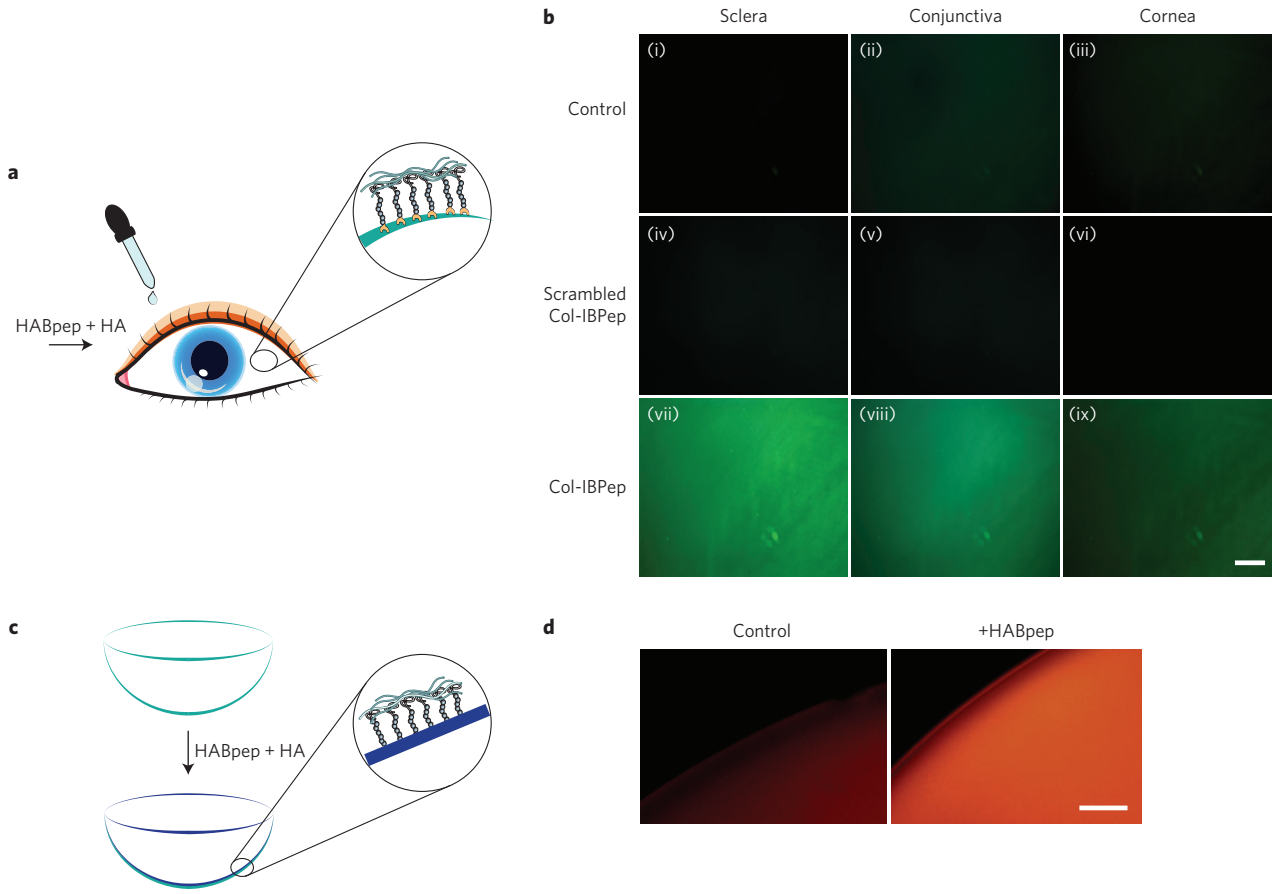

Figure 4 | Ocular surface application of the HABpep–polymer system. a, The HABpep–polymer system as an eye-drop solution can be used to retain HA on the eye surface. Collagen-I-abundant eye tissues without epithelial layers, such as sclera, conjunctiva and cornea, act as anchors for the HABpep–polymer system. **b**, Fluorescence images of HA retention on untreated and treated eye tissues: sclera, conjunctiva and cornea of untreated eye (i–iii); treated with scrambled collagen I binding peptide Col-IBPep; (YFDEYSLSQS; iv–vi); and treated with collagen I binding peptide (vii–ix). Scale bar, 250 μm. **c**, Contact lens modification with the HABpep–polymer system was performed by the covalent reaction methodology. **d**, Fluorescence images for HA–rhodamine retention on a modified contact lens showed relatively darker staining compared with the control. Scale bar, 200 μm.

(Supplementary Information and Supplementary Fig. 5). Prophylactic treatment with HA-binding coatings during trauma treatment may also be able to enhance local surface lubrication and prevent or reduce the onset of joint degeneration.

Ocular applications

HA is a key molecule in many tissues, and its therapeutic application is extending to other fields, including ophthalmology[45]. HA is an important component of artificial tears to treat dry eye and in eye drops that accelerate healing after surgery or trauma[45,46]. Many ophthalmic products, including multipurpose contact-lens care solutions take advantage of HA's ability to enhance wettability and water retention[46,47], which is much needed in treating dry-eye disorders. Furthermore, HA provides several biological benefits[45] to ocular tissues, such as improving corneal epithelial-cell migration and wound healing[48], reducing inflammation[49] and protecting cells from free-radical damage[20]. Therefore, we investigated the application of the HA-binding strategy to ocular tissues, such as sclera, conjunctiva and cornea of an eye, and to medical devices, such as contact lenses. We applied HA bound to HABpep–polymer as an eye drop with the polymer–peptide system anchored onto Type I collagen of the sclera, conjunctiva and cornea of an eye through collagen I binding peptide (Fig. 4a). Fluorescently labelled HA exposed to the treated ocular surface tissues demonstrated stronger binding compared with a scrambled peptide and control untreated tissues, with the sclera showing the highest levels of binding (Fig. 4b). This eye-drop methodology can recruit and retain HA through HABpep functionalization on eye surfaces with a damaged epithelial layer. Extending the technology to a synthetic device, a contact lens surface was covalently modified with the HABpep–polymer system (Fig. 4c). Fluorescently labelled HA through HABpep was visualized to be bound to the lens, confirming the presence of the surface coating (Fig. 4d). As a functional test for HA binding on contact lenses, the rate of water evaporation from lenses was evaluated. The water evaporation rate decreased significantly on the coated contact lens ($0.23 \pm 0.026\,\mu l\,min^{-1}$) compared with a bare contact lens ($0.50 \pm 0.017\,\mu l\,min^{-1}$) and a control contact lens with only physically adsorbed HA ($0.39 \pm 0.033\,\mu l\,min^{-1}$).

Methods

Synthesis of HA-binding peptide. Thiolated HA-binding peptide (C-HABpep; sequence CRRDDGAHWQFNALTVR) was synthesized using standard Fmoc-mediated solid-phase peptide synthesis on a Symphony Quartet peptide synthesizer (Protein Technologies). Following synthesis, peptides were cleaved using a solution of trifluoroacetic acid, triisopropylsilane and water in a 95:2.5:2.5 ratio. Crude product was purified using reverse-phase high-performance liquid chromatography (C18 Grace-Vydac column) on a water/acetonitrile gradient. Purified peptides were frozen and lyophilized; identity of purified peptides was confirmed using matrix-assisted laser-desorption ionization time-of-flight mass spectroscopy.

Preparation of HA-binding coatings. C-HABpep was conjugated to articular cartilage through a heterobifunctional PEG spacer. MAL–PEG–NHS (3.5 kDa),

which has functionalities that are thiol- and amine-reactive, was dissolved to 5 mM in 50 mM sodium bicarbonate, pH 7.5, and added to the articular surface. The NHS groups were allowed to react with endogenous amines on the cartilage surface for 30 min. PEGylation was confirmed by ATR-FTIR. Following thorough washes in buffer to remove unreacted crosslinker, a 1.5 mM solution of C-HABpep was added to the surface to react with maleimide groups for an additional 30 min. Surfaces were carefully washed to remove unreacted peptide, yielding a cartilage surface with covalently attached HA-binding functionality. In another methodology for one-step application, thiol–PEG–succinimidyl glutaramide (SGA; 3.5 kDa) was reacted with HABpep (GAHWQFNALTVR; dissolved in dimethylsulphoxide) in a PBS buffer (pH 7.4) for 4 h. After dialysis and lyophilization, thiol functionality of the product was reacted overnight with vinyl functional groups of vinyl dimethyl azlactone using a Michael-addition reaction in the presence of dimethylphenylphosphine in dimethylsulphoxide at room temperature. The product was dissolved in water and washed multiple times with cold ether and dried *in vacuo*. The resultant product was added to a sodium bicarbonate solution (pH 8.3) of collagen binding peptide (Col II-WRYGRLC, Col I-YSFYSESLQ; refs 50–52). After 4 h of reaction time, the solution was dialysed against water (molecular weight cutoff 2,000 Da) and lyophilized to yield a white fluffy powder.

X-ray photoelectron spectroscopy. XPS was performed to verify the presence of the HA-binding coating on articular cartilage. Lyophilized cartilage samples were adhered to the specimen stage and loaded into a PHI 5400 XPS instrument at ultrahigh vacuum. The samples were analysed using Mg Kα X-rays (1,253.6 eV), and spectra were acquired at a take-off angle of 45°. Atomic concentrations were determined by integration of the relevant photoelectron peaks using commercially available software (CasaXPS).

Visualization of the HA-bound layer. Cartilage was conjugated with C-HABpep and incubated with biotinylated HA synthesized as previously described. Briefly, HA (975 kDa, LifeCore Biomedical) was dissolved in 50 mM boric acid, pH 5.2, at 2.0 mg ml^{-1}. This was combined with biotin hydrazide in a 20:1 weight ratio. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, was added to a final concentration of 100 mM). The reaction was allowed to proceed at room temperature for 16 h, after which the product was dialysed to remove unreacted biotin hydrazide and EDC. HA–biotin was lyophilized and stored at −20 °C for later use. Following incubation with 5 mg ml^{-1} HA–biotin, HABpep-functionalized cartilage samples were washed vigorously to remove unbound HA–biotin. Presence of biotin was visualized using streptavidin and horseradish peroxidase following the methods of the Histostain SP kit (Life Technologies).

Cartilage sample preparation for lubrication testing. Bovine (12–14 weeks old from Research87) and human articular cartilage samples (National Disease Resource Institution) collected from femoral condyles of cartilage were prepared for lubrication testing as a modification of previously published protocols[9,53]. Covalent modification of cartilage surface by HABpep (Fig. 1) was performed on bovine articular cartilage samples. All non-covalent HABpep modification and *in vitro* lubrication studies were performed using human articular cartilage samples (Fig. 3). Normal human tissue was isolated from cadavers with ages 51 (female (F)) and 78 (male (M)) years. Human osteoarthritic cartilage samples were isolated from the patients with ages, 58 (F), 60 (F), 61 (M), 65 (M), 69 (M), 71 (F), who underwent total knee arthroplasty. Care was taken to avoid damaging the articular surface during dissection. Samples (outer diameter = 8.0 mm, inner diameter = 3.0 mm) were microtomed and evenly cut to obtain a flat surface. The superficial layer was maintained intact and only the deep layer of cartilage was cut to obtain a flat layer to glue to the metal counter-surface while performing friction measurements. Cartilage was used fresh without freezing or the addition of protease inhibitors so as not to change the surface lubrication properties. Samples were washed vigorously in PBS overnight to deplete the cartilage surface of any residual synovial fluid, after which they were functionalized with a HA-binding layer as needed and incubated at 4 °C for ∼24 h in the test lubricant. HABpep-modified cartilage samples were soaked overnight in HA bath (5.0 mg ml^{-1}, 975 kDa) followed by washing them vigorously in PBS overnight to remove unbound HA. These samples were incubated in PBS for 1 h and lubrication testing was performed. Cartilage samples with no HABpep modification were incubated in either PBS or HA (5.0 mg ml^{-1}, 975 kDa) for 1 h, after 24 h PBS wash, and lubrication testing was performed. Further experimental information is provided in the Supplementary Information and Supplementary Fig. 3. The friction values were recorded as mean and standard deviation. See Supplementary Table 1a,b for examples of raw data and calculations.

Friction measurements on human cartilage samples without lubricin. Lubricin was removed from the human cartilage samples in accordance with a published procedure[54]. In brief, endogenous lubricin was extracted from cartilage discs on incubation at room temperature for 20 min in PBS containing 1.5 M NaCl followed by an additional 20 min incubation in pH 6.2 of 4 M guanidine-HCl solution. The cartilage discs in each solution were shaken throughout the experiment. Friction measurements on these samples were performed by a procedure as described above.

***In vivo* imaging for HA retention.** *In vivo* imaging was conducted on 6–8-week-old male Sprague Dawley rats (n = 4 for each group; total = 8). The rats were anaesthetized with isoflurane under a pre-established protocol (The Johns Hopkins University Animal Care and Use Committee approved the animal procedures, protocol number RA12A136). Each rat was injected with a 50 µl solution mixture of HA–rhodamine (20 mg ml^{-1}, CreativePEGworks) with HABpep–polymer (10 mg ml^{-1}) under sterile conditions penetrating the joint capsule and bursa. After injection, rats were imaged and kept under isoflurane anaesthesia with an IVIS Spectrum *in vivo* imaging system (rats were imaged at different time points: immediately after surgery (<2 h), 6 h, 24 h and 72 h after surgery). All images were taken at the same excitation (570 nm) and emission (620 nm). Rats were anaesthetized before each imaging time point. A method for semi-quantitative analysis of HA bound on the cartilage surface is described in the Supplementary Information.

Ocular surfaces and contact lens modification. Rabbit eyes (8–12-week-old rabbits purchased from Pel-Freez) were processed *ex vivo* to separate epithelial layers from the sclera, conjunctiva and cornea. Each of these tissues was cut into small pieces using a 3-mm-diameter biopsy punch, and washed rigorously with PBS. To these tissues, a solution of HA–fluorescein (975 kDa, CreativePEGworks), mixed with HABpep–PEG–Col IBpep polymer was added (final concentration 5.0 and 1.0 mg ml^{-1}, respectively), kept on a shaker for 2 h and washed with PBS before taking images by Zeiss Discovery V2 imaging microscope. The ability of the HABPep technology to bind HA to multiple different external ocular tissues was tested and compared with controls for each tissue using fluorescein-labelled HA. Tissue samples were cut from biopsy punches and separated into three equal pieces and treated with HA alone (negative control for nonspecific HA binding), HA with scramble peptide (control for nonspecific peptide binding) and HA with Col IBpep). All ocular tissues were imaged at the same exposure time and magnification. Contact lens (PureVision from Bausch & Lomb was treated with diazirine-based photo-leucine (5.0 mg ml^{-1}) under ultraviolet light (365 nm) for 30 min at an approximate distance of 3.0 cm, followed by reacting the amine groups with azlactone functionality of the PEG–HABpep (1.0 mg ml^{-1}) in a sodium bicarbonate solution (pH 8.3) for 4 h. HABpep-modified contact lenses were added to a solution of HA–rhodamine (1.0 mg ml^{-1}) and kept on a shaker for 2 h. After washing with PBS, fluorescence images were taken by a Zeiss Discovery V2 imaging microscope and processed with ImageJ. To measure water evaporation rate from the contact lens, an evaporation cell was designed by cutting the cap and hinge off a 1.5 ml SealRite microcentrifuge tube (USA Scientific). After filling the cell with 1.2 ml of Hank's buffer solution (HBSS), the contact lens was glued with instant Krazy Glue (Elmer's Products) to the rim of the cell. The cell was gently placed on its side, keeping the contact lens inside completely hydrated, into an analytical balance and the weight of the cell was recorded at the start and every 5 min for 50 min (n = 3).

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References

1. Dorinson, A. & Ludema, K. C. *Mechanics and Chemistry in Lubrication*, Ch. 1 (Elsevier, 1985).
2. Kato, K. Industrial tribology in the past and future. *Tribology* **6,** 1–9 (2011).
3. Moghani, T., Butler, J. P. & Loring, S. H. Determinants of friction in soft elastohydrodynamic lubrication. *J. Biomech.* **42,** 1069–1074 (2009).
4. Chawla, K., Ham, H. O., Nguyen, T. & Messersmith, P. B. Molecular resurfacing of cartilage with proteoglycan 4 (PRG4). *Acta Biomater.* **6,** 3388–3394 (2010).
5. Flannery, C. R. *et al.* Prevention of cartilage degeneration in a rat model of osteoarthritis by intracellular treatment with recombinant lubricin. *Arthritis Rheum.* **60,** 840–847 (2009).
6. McNary, S. M., Athanasiou, K. A. & Reddi, A. H. Engineering lubrication in articular cartilage. *Tissue Eng. Part B Rev.* **18,** 88–100 (2012).
7. Sivan, S. *et al.* Liposomes act as effective biolubricants for friction reduction in human synovial joints. *Langmuir* **26,** 1107–1116 (2010).
8. Hills, B. A. & Butler, B. D. Surfactants identified in synovial fluid and their ability to act as boundary lubricants. *Ann. Rheum. Dis.* **43,** 641–648 (1984).
9. Schmidt, T. A., Gastelum, N. S., Nguyen, Q. T., Schumacher, B. L. & Sah, R. L. Boundary lubrication of articular cartilage: Role of synovial fluid constituents. *Arthritis Rheum.* **56,** 882–891 (2007).
10. Neu, C. P., Komvopoulos, K. & Reddi, A. H. The interface of functional biotribology and regenerative medicine in synovial joints. *Tissue Eng. Part B Rev.* **14,** 235–247 (2008).

11. Chen, M., Briscoe, W. H., Armes, S. P. & Klein, J. Lubrication at physiological pressures by polyzwitterionic brushes. *Science* **323,** 1698–1701 (2009).
12. Schmidt, T. A. *et al.* Transcription, translation, and function of lubricin, a boundary lubricant, at the ocular surface. *JAMA Ophthalmol.* **131,** 766–776 (2013).
13. Zmolik, J. M. & Mummert, M. E. Pep-1 as a novel probe for the *in situ* detection of hyaluronan. *J. Histochem. Cytochem.* **53,** 745–751 (2005).
14. Mummert, M. E., Mohamadzadeh, M., Mummert, D. I., Mizumoto, N. & Takashima, A. Development of a peptide inhibitor of hyaluronan-mediated leukocyte trafficking. *J. Exp. Med.* **192,** 769–780 (2000).
15. Tolg, C. *et al.* A RHAMM mimetic peptide blocks hyaluronan signaling and reduces inflammation and fibrogenesis and excisional skin wounds. *Am. J. Pathol.* **181,** 1250–1270 (2012).
16. Zaleski, K. J. *et al.* Hyaluronic acid binding peptides prevent experimental staphylococcal wound infection. *Antimicrob. Agents Chemother.* **50,** 3856–3860 (2006).
17. Yang, B., Zhang, L. & Turley, E. A. Identification of two hyaluronan-binding domains in the hyaluronan receptor RHAMM. *J. Biol. Chem.* **268,** 8617–8623 (1993).
18. Jay, G. D., Torres, J. R., Warman, M. L., Laderer, M. C. & Breuer, K. S. The role of lubricin in the mechanical behavior of synovial fluid. *Proc. Natl Acad. Sci. USA* **104,** 6194–6199 (2007).
19. Zhang, D., Johnson, L. J., Hsu, H. P. & Spector, M. Cartilaginous deposits in subchondral bone in regions of exposed bone in osteoarthritis of the human knee: Histomorphometric study of PRG4 distribution in osteoarthritic cartilage. *J. Orthop. Res.* **25,** 873–883 (2007).
20. Presti, D. & Scott, J. E. Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl (OH.) radicals is dependent on hyaluronan molecular mass. *Cell Biochem. Funct.* **12,** 281–288 (1994).
21. Julovi, S. M., Yasuda, T., Shimizu, M., Hiramitsu, T. & Nakamura, T. Inhibition of interleukin-1β-stimulated production of matrix metalloproteinases by hyaluronan via CD44 in human articular cartilage. *Arthritis Rheum.* **50,** 516–525 (2004).
22. Fraser, J. R. E., Laurent, T. C. & Laurent, U. B. G. Hyaluronan: Its nature, distribution, functions and turnover. *J. Intern. Med.* **242,** 27–33, (1997).
23. Buckwalter, J. A. & Mankin, H. J. Articular cartilage. Part II: Degeneration and osteoarthrosis, repair, regeneration, and transplantation. *J. Bone Joint Surg. Am.* **79,** 612–632 (1997).
24. Morrell, K. C., Hodge, W. A., Krebs, D. E. & Mann, R. W. Corroboration of *in vivo* cartilage pressures with implications for synovial joint tribology and osteoarthritis causation. *Proc. Natl Acad. Sci. USA* **102,** 14819–14824 (2005).
25. Greene, G. W. *et al.* Adaptive mechanically controlled lubrication mechanism found in articular joints. *Proc. Natl Acad. Sci. USA* **108,** 5255–5259 (2011).
26. Das, S. *et al.* Synergistic interactions between grafted hyaluronic acid and lubricin provide enhanced wear protection and lubrication. *Biomacromolecules* **14,** 1669–1677 (2013).
27. Amiel, D. *et al.* Long-term effect of sodium hyaluronate (Hyalgan®) on osteoarthritis progression in a rabbit model. *Osteoarthritis Cartilage* **11,** 636–643 (2003).
28. Yoshimi, T. *et al.* Effects of high-molecular-weight sodium hyaluronate on experimental osteoarthrosis induced by the resection of rabbit anterior cruciate ligament. *Clin. Orthop. Relat. Res.* **298,** 296–304 (1994).
29. Elmory, S. *et al.* Chondroprotective effects of high-molecular-weight cross-linked hyaluronic acid in a rabbit knee osteoarthritis model. *Osteoarthritis Cartilage* **22,** 121–127 (2014).
30. Yu, C-J. *et al.* Proteomic analysis of osteoarthritic chondrocyte reveals the hyaluronic acid regulated proteins involved in chondroprotective effect under oxidative stress. *J. Proteomics* **99,** 40–53 (2014).
31. Moreland, L. W. Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: Mechanisms of action. *Arthritis Res. Ther.* **5,** 54–67 (2003).
32. Sharma, B., Williams, C. G., Khan, M., Manson, P. & Elisseeff, J .H. *In vivo* chondrogenesis of mesenchymal stem cells in photopolymerized hydrogel. *Plast. Reconstr. Surg.* **119,** 112–120 (2007).
33. Jackson, D. W. & Simon, T. M. Intra-articular distribution and residence time of Hylan A and B: A study in the goat knee. *Osteoarthritis Cartilage* **14,** 1248–1257 (2006).
34. Zhang, W. *et al.* OARSI recommendations for the management of hip and knee osteoarthritis: Part III: Changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthritis Cartilage* **18,** 476–499 (2010).
35. Strauss, E. J., Hart, J. A., Miller, M. D., Altman, R. D. & Rosen, J. E. Hyaluronic acid viscosupplementation and osteoarthritis: Current uses and future directions. *Am. J. Sports Med.* **37,** 1636–1644 (2009).
36. Brandt, K. D., Smith, G. N. & Simon, L. S. Intraarticular injection of hyaluronan as treatment for knee osteoarthritis: What is the evidence. *Arthritis Rheum.* **43,** 1192–1203 (2000).
37. Sharma, B. *et al.* Human cartilage repair with a photoreactive adhesive-hydrogel composite. *Sci. Transl. Med.* **5,** 167ra6 (2013).
38. Wang, D. A. *et al.* Multifunctional chondroitin sulphate for cartilage tissue-biomaterial integration. *Nature Mater.* **6,** 385–392 (2007).
39. Messman, J. M., Lokitz, B. S., Pickel, J. M. & Kilbey, S. M. Highly tailorable materials based on 2-vinyl-4,4-dimethyl azlactone: (co)polymerization, synthetic manipulation and characterization. *Macromolecules* **42,** 3933–3941 (2009).
40. Brown, T. J., Laurent, U. B. G. & Fraser, J. R. E. Turnover of hyaluronan in synovial joints: Elimination of labeled hyaluronan from the knee joint of the rabbit. *Exp. Physiol.* **76,** 125–134 (1991).
41. Smith, G. N., Mickler, E. A., Myers, S. L. & Brandt, K. D. Effect of intraarticular hyaluronan injection on synovial fluid hyaluronan in the early stage of canine post-traumatic osteoarthritis. *J. Rheum.* **28,** 1341–1346 (2001).
42. Caligaris, M., Canal, C. E., Ahmad, C. S., Gardner, T. R. & Ateshian, G. A. Investigation of the frictional response of osteoarthritic human tibiofemoral joints and the potential beneficial tribological effect of healthy synovial fluid. *Osteoarthritis Cartilage* **17,** 1327–1332 (2009).
43. Lee, S. S. *et al.* Frictional response of normal and osteoarthritic articular cartilage in human femoral head. *Proc. Inst. Mech. Eng. H.* **227,** 129–137 (2013).
44. Desrochers, J., Amrein, M. W. & Matyas, J. R. Microscale surface friction of articular cartilage in early osteoarthritis. *J. Mech. Behav. Biomed. Mater.* **25,** 11–22 (2013).
45. Rah, M. J. A review of hyaluronan and its ophthalmic applications. *Optometry* **82,** 38–43 (2011).
46. Fonn, D. Targeting contact lens induced dryness and discomfort: What properties will make lenses more comfortable. *Optom. Vis. Sci.* **84,** 279–285 (2007).
47. Hargittai, I. & Hargittai, M. Molecular structure of hyaluronan: An introduction. *Struct. Chem.* **19,** 697–717 (2008).
48. Inoue, M. & Katakami, C. The effect of hyaluronic acid on corneal epithelial cell proliferation. *Invest. Opthalmol. Vis. Sci.* **34,** 2313–2315 (1993).
49. Pauloin, T., Dutot, M., Joly, F., Warnet, J. M. & Rat, P. High molecular weight hyaluronan decreases UVB-induced apoptosis and inflammation in human epithelial corneal cells. *Mol. Vis.* **15,** 577–583 (2009).
50. Rothenfluh, D. A., Bermudez, H., O'Neil, C. P. & Hubbell, J. A. Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. *Nature Mater.* **7,** 248–254 (2008).
51. Löster, K., Zeilinger, K., Schuppan, D. & Reutter, W. The cysteine-rich region of dipeptidyl peptidase IV (CD26) is the collagen-binding site. *Biochem. Biophys. Res. Commun.* **217,** 341–348 (1995).
52. Sistiabudi, R. & Ivanisevic, A. Collagen-binding peptide interaction with retinal tissue surfaces. *Langmuir* **24,** 1591–1594 (2008).
53. Schmidt, T. A. & Sah, R. L. Effect of synovial fluid on boundary lubrication of articular cartilage. *Osteoarthritis Cartilage* **15,** 35–47 (2007).
54. Jones, A. R. *et al.* Binding and localization of recombinant lubricin to articular cartilage surfaces. *J. Orthop. Res.* **25,** 283–292 (2007).

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Author contributions

The experiments were designed by A.S., M.C., S.A.U., P.M. and J.H.E., and carried out by A.S., M.C., S.A.U. and K.A.W. Data analyses were performed by A.S., M.C. and J.H.E. The manuscript was written by A.S. and J.H.E.

Additional information

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Competing financial interests

The authors declare no competing financial interests.